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Directional transfer of a multiple-allele male sterile line in *Brassica campestris* L. ssp. *chinensis* (L.) Makino var. *rosularis* Tsen et Lee

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To produce hybrid seeds of Wutacai (*Brassica campestris* L. ssp. *chinensis* (L.) Makino var. *rosularis* Tsen et Lee), a "directional transfer program" was designed to breed the multiple-allele male sterile line of Wutacai. A multiple-allele male sterile line of Naibaicai (*Brassica campestris* L. ssp. *chinensis* L., S01) was used as the male sterile resource, and an inbred line of Wutacai (WT01) was used as the target line. Recurrent backcrossing was employed to transfer the male sterility and other botanical traits simultaneously, while the genotype was identified through test crossing. The male sterility was successfully transferred from S01 to WT01. A new male sterile line, GMS-3, with similar botanical traits to WT01, was bred. Four hybrid combinations were generated with GMS-3 as the female parent. One hybrid (C1) that contained the most desirable traits was developed from the new male sterile line.

Key Words: *Brassica campestris* L. ssp. *chinensis* (L.) Makino var. *rosularis* Tsen et Lee, multiple-allele male sterile line, breeding.

Introduction

Wutacai, a variant of the subspecies of *Brassica campestris*, originated in China and is widely distributed in the Yangtze River basin (Li 1990). Its glossy dark-green leaflets have many folds and its rosette leaves are arranged in wheel-like growth forms. It has been given the laudatory name "leaf peony" because of its beautiful shape. Wutacai tastes fresh and crisp, and is also called the "vitamin" vegetable (Li 2000b) due to its high ascorbic acid content and the presence of other trace nutrients, such as carotene, calcium, iron, phosphorus, and zinc (Shu and Zhou 2005).

Wutacai is an example of an allogamous plant with bisexual flowers and obvious heterosis (Feng *et al.* 2008). However, due to a lack of ideal hybrid seed production procedures, only conventional varieties are currently available. Male sterility is an important approach to exploiting heterosis (Havey 2004, Ke *et al.* 1992, Zhang *et al.* 1990). Through this approach, not only can a high level of purity be obtained in hybrid seeds, but the intellectual property of breeders can also be protected. As reported by Xu *et al.* (2007), Ogura cytoplasmic male sterility in Chinese cabbage can be successfully transferred to Wutacai. However, the disadvantages of this transfer include leaf etiolation, nectar degeneration, and a slowed growth rate, such that the method cannot be extensively utilized for hybrid seed production. Feng *et al.* (1995) found an incidence of multiple-allele male sterility in Chinese cabbage and proposed the "genetic hypothesis of multiple-allele male sterile gene in Chinese cabbage". Based on this hypothesis, male sterility is dominated by a multiple-allele locus that includes Ms^f, Ms, and ms. Among them, the Ms allele is a male sterile gene, the ms allele is a fertile gene, and the Ms^f allele is a fertility restoration gene. The dominant to recessive relationship of the alleles is $Ms^f > Ms > ms$. The male sterile AB line in this hypothesis is used to maintain male sterile plants. It contains two genotypes of Ms^fMs and $MsMs$, half of which is fertile and the other half is sterile. The AB line is maintained by sib mating between the male sterile and fertile plants ($MsMs \times Ms^fMs \rightarrow 1/2\ MsMs,\ 1/2\ Ms^fMs$) (Fig. 2). This male sterility is characterized by its stable and complete sterile performance without a negative cytoplasmic effect. Xu *et al.* (2011) reported that the multiple-allele male sterility of pak choi (*Brassica campestris* L. ssp. *chinensis* L.) was transferred to Wutacai through crossing and bred a male sterile line. However, this male sterile line was developed from two parents showing significant differences in their genetic backgrounds. Therefore, the F_1 hybrids that developed from the male sterile line are not uniform.

The current research aimed to find an appropriate method for breeding the multiple-allele male sterile line of Wutacai. To achieve this objective, a new multiple-allele male sterile line of Naibaicai was adopted, and a "directional transfer program" was designed.

Materials and Methods

Plant materials

All materials used in this research were provided by the Liaoning Key Laboratory of Genetics and Breeding of

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Fig. 1. Photographs of transfer materials and hybrid combinations. Explanation of plates: A. S01, a male sterile line of Naibaicai; B. inflorescence of S01; C. TW01, target line of Wutacai; D. inflorescence of TW01; E. GMS-3, a male sterile line of Wutacai; F. inflorescence of GMS-3; G. hybrid combinations of Wutacai; H. C1, a hybrid combination of Wutacai.

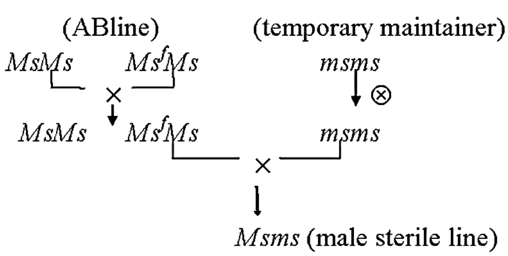

Fig. 2. Genetic model for the multiple-allele inherited male sterile line in Chinese cabbage.

Cruciferous Vegetable Crops in China. Male sterility resources and testcross materials were from S01, a male sterile line of Naibaicai (Fig. 1A, 1B). The target line WT01 is an excellent inbred line of Wutacai and was used as a recurrent parent (Fig. 1C, 1D). Hybrid varieties were made by crosses between a new male sterile line and four high-quality and stable genetic inbred lines (P_1, P_2, P_3, and P_4), and their performances were compared.

Conventional transformation methods

Our experiment was conducted at the Horticulture Research Laboratory of Shenyang Agricultural University from 2008 to 2011. Two sexual generations were grown each year. In spring, sowing and seedling growth were completed in a sunlight greenhouse. When the true leaves grew to 6–7 films, the seedlings were transplanted into 22-cm mud pots. At the end of May, inflorescences were covered with pollination bags, and pollination was carried out by hand when the plants were flowering. A second generation was added in autumn and winter. Seeds were vernalized after germination at 2°C in a refrigerator for 25 days. Without being transplanted, plant materials of test crosses were directly sowed in a tray with holes after vernalization, and fertility rates were determined when the plants bolted and flowered. Crossing, backcrossing, test crossing, and selfing were employed to transfer the male sterility.

The assessment of sterility

To assess "the degree of male sterility" in Wutacai (GMS-3), 1000 flowers from 100 plants (keeping 10 flowers per inflorescence per plant and removing the other flowers) were covered with pollination bags to prevent pollination, and their selfed seed fertility was examined. The number of flowers inside pollination bags that resulted in seedpods was recorded at maturity. To assess "the percentage of male sterile plants", 100 GMS-3s were planted and the fertility of individual plants (the number of plants with pollen in flowers) was recorded at flowering.

Comparative experiment

Plots were arranged in the field in a randomized complete block design with three replicates. Each of the plots measured 3 m × 1.5 m. Plant and row spacing was 30 cm × 50 cm. With the newly bred GMS-3 as the female parent, four combinations were generated by crossing GMS-3 with the four inbred lines: P_1, P_2, P_3, and P_4. The 'vitamin' variety was used as a control (CK).

Chemical components analysis

Samples (leaves) of plants were collected at random from each plot (four plants per plot) during harvest and then mixed into composite samples to carry out the analysis. Several chemical measurements were taken from leaves: ascorbic acid content, soluble sugar content, protein content, organic acid content, crude fiber content, and the amount of six trace elements. The ascorbic acid component was measured by molybdenum blue colorimetry (Li 2000a). The anthrone colorimetry method was adopted to determine the content of soluble sugars (Shi *et al.* 2011). Coomassie brilliant blue G 250 staining was used for the detection of proteins (Liu and Lin 2008). Organic acid content was determined by acid-base titration, and the acid washing method was used for crude fiber content analysis (Xie and Qu 2006). Atomic absorption spectrometry was adopted to analyze for trace elements content (Deng 2003).

Results

Genotyping of the target line

Based on the "genetic hypothesis of multiple-allele male sterile gene in Chinese cabbage," the special genetic model of this locus results in six genotypes, of which four genotypes, Ms^fMs^f, $msms$, Ms^fms, and Ms^fMs, shared an identical fertile phenotype. The genotype Ms^fMs was eliminated in the breeding process because its selfing progenies displayed a 3 : 1 segregation ratio for fertility and sterility. The remaining three genotypes were distinguished by a test cross. Fig. 3 shows the model of identifying the genotypes. In this research, 45 fertile F_1 plants were obtained from the cross between male sterile plants in the AB line of Naibaicai and WT01, indicating that the genotype of WT01 was Ms^fMs^f.

The breeding model

The genetic model of directional transfer was designed in accordance with the results of genotype identification of the target line. The line S01 with the $Msms$ genotype was selected as a male sterility resource, and the target line WT01 with the Ms^fMs^f genotype was used as the recurrent parent. The "AB line direction" represents the populations in backcross generations that were selected to screen the AB line, whereas the "temporary maintainer direction" indicates a population that was selected to screen for a temporary maintainer (Fig. 4).

$$MsMs \times \begin{cases} Ms^fMs^f \rightarrow Ms^fMs & \text{all fertile} \\ Ms^fms \rightarrow Ms^fMs, Msms & \text{1:1 (fertile: sterile)} \\ msms \rightarrow Msms & \text{all sterile} \end{cases}$$

Fig. 3. Genotype identification of the target line TW01.

The breeding results

According to the model shown in Fig. 3, F_1 plants should have two genotypes (Ms^fMs, Ms^fms) showing an identical fertile phenotype. Seven plants were selected and then self-pollinated to obtain the F_2 generation. The genotypes of F_1 plants could be identified according to the segregation ratio for fertility and sterility in the F_2 population (Table 1).

Simultaneously, a backcross was conducted between the seven F_1 plants and the recurrent parent to construct a BC_1 population. The BC_1 plants used for the AB line direction (Ms^fMs) and temporary maintainer direction (Ms^fms) were distinguished from each other according to the results of genotype identification of F_1 plants (Table 2). In the next generation, seven plants were selected from each direction of BC_1 to conduct a backcross with the recurrent parent and to obtain a BC_2 generation. At the same time, these plants

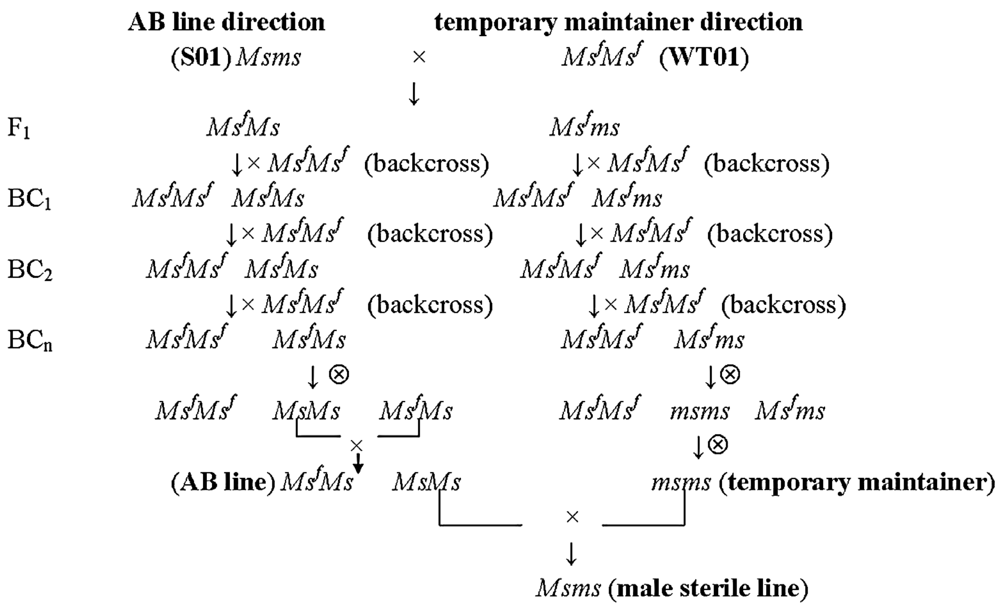

Fig. 4. Genetic model for the directional transfer of the multiple-allele male sterile line in Wutacai.

Table 1. Segregation ratio for fertility and sterility in the F_2 generation

Code	Fertile plants/Sterile plants	Theoretical ratio ($X^2_{0.05,1}$ = 3.84)	Genotype of F_1 plant	Genotypes of F_2 population
(S01 × WT01)-1⊗	50/0	All fertile	Ms^fms	Ms^fMs^f, Ms^fms, $msms$
(S01 × WT01)-2⊗	35/13	3 : 1 (0.111)	Ms^fMs	Ms^fMs^f, Ms^fMs, $MsMs$
(S01 × WT01)-3⊗	36/12	3 : 1 (0.000)	Ms^fMs	Ms^fMs^f, Ms^fMs, $MsMs$
(S01 × WT01)-4⊗	39/14	3 : 1 (0.057)	Ms^fMs	Ms^fMs^f, Ms^fMs, $MsMs$
(S01 × WT01)-5⊗	46/0	All fertile	Ms^fms	Ms^fMs^f, Ms^fms, $msms$
(S01 × WT01)-6⊗	49/0	All fertile	Ms^fms	Ms^fMs^f, Ms^fms, $msms$
(S01 × WT01)-7⊗	39/16	3 : 1 (0.491)	Ms^fMs	Ms^fMs^f, Ms^fMs, $MsMs$

Table 2. Identification of the AB line direction and temporary maintainer direction in BC_1

Code of BC_1	Transfer direction	Genotype of F_1 plant	Genotype of BC_1 plant
(S01 × WT01)-1 × WT01	TM[a] direction	Ms^fms	$Ms^fms \times Ms^fMs^f$
(S01 × WT01)-2 × WT01	AB line direction	Ms^fMs	$Ms^fMs \times Ms^fMs^f$
(S01 × WT01)-3 × WT01	AB line direction	Ms^fMs	$Ms^fMs \times Ms^fMs^f$
(S01 × WT01)-4 × WT01	AB line direction	Ms^fMs	$Ms^fMs \times Ms^fMs^f$
(S01 × WT01)-5 × WT01	TM direction	Ms^fms	$Ms^fms \times Ms^fMs^f$
(S01 × WT01)-6 × WT01	TM direction	Ms^fms	$Ms^fms \times Ms^fMs^f$
(S01 × WT01)-7 × WT01	AB line direction	Ms^fMs	$Ms^fMs \times Ms^fMs^f$

[a] Temporary maintainer.

were test crossed with S01. The backcross progenies of the BC_1 plants were identified as Ms^fMs and Ms^fms, meaning that the AB line direction and the "temporary maintainer" direction of the BC_2 line could be developed, while those of the Ms^fMs^f plants were eliminated (Table 3). Fig. 5 shows the genetic model of the test crosses. By repeating this procedure, the AB line direction and the temporary maintainer direction of BC_3 and BC_4 could also be developed.

In Table 4, we present the results of genotype identification of selected plants in each backcross generation. Plants resulting from the test cross that were close to the theoretical segregation ratio and those with desired botanical traits were selected. After four generations of backcrossing, seven plants were selected from each direction and were both self- and test-crossed. According to the results from the test cross, the plants identified as Ms^fMs and Ms^fms were selected and their selfing progenies were selected as target groups to screen the AB line and the temporary maintainer. The selected groups are shown in Table 5.

Table 3. Genotype identification results of Ms^fMs and Ms^fms in the BC_2 generation

Code	Fertile plants/ Sterile plants	Theoretical ratio ($X^2_{0.05,1} = 3.84$)	Genotype of plant
S01 × (BC_1-4 × WT01)-1	49/0	All fertile	Ms^fMs^f
S01 × (BC_1-4 × WT01)-2	23/27	1 : 1 (0.320)	Ms^fMs
S01 × (BC_1-4 × WT01)-3	46/0	All fertile	Ms^fMs^f
S01 × (BC_1-4 × WT01)-4	42/0	All fertile	Ms^fMs^f
S01 × (BC_1-4 × WT01)-5	20/27	1 : 1 (1.160)	Ms^fMs
S01 × (BC_1-4 × WT01)-6	22/26	1 : 1 (0.400)	Ms^fMs
S01 × (BC_1-4 × WT01)-7	49/0	All fertile	Ms^fMs^f
S01 × (BC_1-5 × WT01)-1	48/0	All fertile	Ms^fMs^f
S01 × (BC_1-5 × WT01)-2	45/0	All fertile	Ms^fMs^f
S01 × (BC_1-5 × WT01)-3	43/0	All fertile	Ms^fMs^f
S01 × (BC_1-5 × WT01)-4	48/0	All fertile	Ms^fMs^f
S01 × (BC_1-5 × WT01)-5	40/17	3 : 1 (0.708)	Ms^fms
S01 × (BC_1-5 × WT01)-6	38/14	3 : 1 (0.103)	Ms^fms
S01 × (BC_1-5 × WT01)-7	46/0	All fertile	Ms^fMs^f

Table 4. Genotype identification of selected plants in each backcross generation during transfer of the male sterile line

Backcross generation	Code of plant	Fertile plants/ Sterile plants	Theoretical ratio ($X^2_{0.05,1} = 3.84$)	Genotype of plant
BC_1	(F_1 × WT01)-4	39/14	3 : 1 (0.057)	Ms^fMs
BC_2	(BC_1 × WT01)-6	22/26	1 : 1 (0.400)	Ms^fMs
BC_3	(BC_2 × WT01)-7	24/22	1 : 1 (0.087)	Ms^fMs
BC_4	(BC_3 × WT01)-6	24/23	1 : 1 (0.021)	Ms^fMs
BC_1	(F_1 × WT01)-5	46/0	All fertile	Ms^fms
BC_2	(BC_1 × WT01)-6	38/14	3 : 1 (0.103)	Ms^fms
BC_3	(BC_2 × WT01)-1	39/14	3 : 1 (0.057)	Ms^fms
BC_4	(BC_3 × WT01)-7	38/15	3 : 1 (0.308)	Ms^fms

In the AB line direction of the target groups, selfing of Ms^fMs resulted in a fertile and sterile plant segregation ratio of 3 : 1. Three genotypes (Ms^fMs^f, Ms^fMs and $MsMs$) were observed in the selfing progenies of Ms^fMs. Sib mating was performed between the male sterile plants ($MsMs$) and five fertile plants (Ms^fMs^f or Ms^fMs) that were randomly selected. The new AB line (Ms^fMs, $MsMs$) was obtained if the progeny shared a 1 : 1 segregation ratio (Table 6). In the temporary maintainer direction of the target groups, three genotypes (Ms^fMs^f, Ms^fms and $msms$) also existed in the selfing progenies of the Ms^fms plants, and all of the plants were fertile. Sixteen fertile plants were selected to perform selfing and test crossing with the male sterile plant ($MsMs$) found in the selfing progeny of the Ms^fMs plants. In this manner, the temporary maintainer was obtained, and all test crossed progenies were sterile (Table 7).

Since the optimal male sterile plant in the AB line was chosen to cross with the temporary maintainer, a male sterile line was obtained, namely GMS-3 (Table 8) (Fig. 1E, 1F).

The assessment of sterility in the male sterile line

One thousand flowers were covered with pollination bags to prevent open pollination. Two types of results were obtained. One result was that the flowers inside the pollination

$$Msms \times \begin{cases} Ms^fMs \rightarrow Ms^fMs, Ms^fms, MsMs, Msms & \text{1:1 (fertile: sterile)} \\ Ms^fMs^f \rightarrow Ms^fMs, Ms^fms & \text{100\% fertile plants} \end{cases}$$

$$Msms \times \begin{cases} Ms^fMs^f \rightarrow Ms^fMs, Ms^fms & \text{100\% fertile plants} \\ Ms^fms \rightarrow Ms^fMs, Ms^fms, Msms, msms & \text{3:1 (fertile: sterile)} \end{cases}$$

Fig. 5. Genetic model for the test cross.

Table 5. Genotype identification of progenies from BC_4 selfing

Transfer direction	Selfing combinations	Test cross results Fertile/Sterile	Theoretical ratio ($X^2_{0.05,1}$ = 3.84)	Genotype of plant
AB line direction	BC_4-6⊗	25/23	1 : 1 (0.083)	*Ms^fMs^f*, *Ms^fMs*, *MsMs*
Temporary maintainer	BC_4-7⊗	39/10	3 : 1 (0.551)	*Ms^fMs^f*, *Ms^fms*, *msms*

Table 6. Genotype identification of sib mating in the AB line direction

Code	Fertile plants/ Sterile plants	Theoretical ratio ($X^2_{0.05,1}$ = 3.84)	Genotype of fertile plant
A-s[a]-1 × B-f[b]-1	25/24	1 : 1 (0.020)	*Ms^fMs*
A-s-1 × B-f-2	27/23	1 : 1 (0.320)	*Ms^fMs*
A-s-1 × B-f-3	49/0	All fertile	*Ms^fMs^f*
A-s-1 × B-f-4	50/0	All fertile	*Ms^fMs^f*
A-s-1 × B-f-5	22/26	1 : 1 (0.333)	*Ms^fMs*

[a] Sterile plants of selfing progenies of BC_4-6.
[b] Fertile plants of selfing progenies of BC_4-6.

Table 7. Genotype identification of the temporary maintainer line direction

Code	Fertile plants/ Sterile plants	Theoretical ratio ($X^2_{0.05,1}$ = 3.84)	Genotype of fertile plant
A-s-2 × (BC_4-7⊗)-1	26/23	1 : 1 (0.184)	*Ms^fms*
A-s-2 × (BC_4-7⊗)-2	50/0	All fertile	*Ms^fMs^f*
A-s-2 × (BC_4-7⊗)-3	29/21	1 : 1 (1.280)	Ms^fms
A-s-2 × (BC_4-7⊗)-4	0/45	All sterile	*msms*
A-s-2 × (BC_4-7⊗)-5	25/22	1 : 1 (0.191)	*Ms^fms*
A-s-2 × (BC_4-7⊗)-6	0/44	All sterile	*msms*
A-s-3 × (BC_4-7⊗)-7	27/26	1 : 1 (0.019)	*Ms^fms*
A-s-3 × (BC_4-7⊗)-8	48/0	All fertile	*Ms^fMs^f*
A-s-3 × (BC_4-7⊗)-9	20/26	1 : 1 (0.783)	*Ms^fms*
A-s-3 × (BC_4-7⊗)-10	22/27	1 : 1 (0.510)	*Ms^fms*
A-s-3 × (BC_4-7⊗)-11	22/26	1 : 1 (0.333)	*Ms^fms*
A-s-4 × (BC_4-7⊗)-12	0/44	All sterile	*msms*
A-s-4 × (BC_4-7⊗)-13	49/0	All fertile	*Ms^fMs^f*
A-s-4 × (BC_4-7⊗)-14	42/0	All fertile	*Ms^fMs^f*
A-s-4 × (BC_4-7⊗)-15	24/27	1 : 1 (0.176)	*Ms^fms*
A-s-4 × (BC_4-7⊗)-16	25/23	1 : 1 (0.083)	*Ms^fms*

Table 8. Genotype identification of the male sterile line of Wutacai

Code	Combination	Fertile plants/ Sterile plants	Theoretical ratio ($X^2_{0.05,1}$ = 3.84)	Genotype of plant
GMS-3	A-s-2 × (BC_4-7⊗)-4	0/45	All sterile	*Msms*

bags did not result in pods. The other result was that the pods did not contain seeds. Another 100 GMS-3s were planted to identify fertility at flowering. All of the 100 GMS-4 plants had no pollen and they showed complete and stable male sterility (Table 9).

Yield analysis of the hybrids developed from the male sterile line

Nine plants were collected per plot at random and the fresh weight of individuals was measured at harvest time. These results are listed in Table 10. The yields of C1 and C4 were significantly higher than CK (at the 1% level), and plot yields for C1 and C4 were 14.82 kg and 15.81 kg, respectively. C3 yielded significantly higher than CK at the 5% level. Concerning the yields, C2 was not significantly different from CK.

Analysis of chemical component in the hybrids developed from the male sterile line

Chemical components of the hybrids were measured three times. The results show an overall high content of chemical components in hybrid combinations of Wutacai lines; however, some indices were significantly lower than those of CK. Among the four combinations, C1 differed the most. C1's protein and ascorbic acid (Vc) contents were significantly higher than those of CK at 1% level. C1's soluble sugar content was also significantly higher than that of CK at the 5% level. The organic acid and crude fiber contents of C1 were significantly lower than those of CK. Moreover, the trace element contents were also higher in C1 (Table 11).

Discussion

Stable and complete sterility of the male sterile line is suitable for cruciferous vegetable breeding in cases when the potential for heterosis is already known (Ke *et al.* 1992). The multiple-allele male sterile line has the required characteristics. Many studies have been carried out for multiple-allele male sterile line breeding and have proven successful in almost all ecotypes of Chinese cabbage (Li *et al.* 2009, Wang *et al.* 2010, Yue and Feng 2005, Zhang *et al.* 2010). This male sterility was also applied to other vegetable crops in subspecies of *Brassica campestris*, which include pak choi (*B. campestris* L. ssp. *chinensis* L.; Feng *et al.* 2007, Wang *et al.* 2011, Xin *et al.* 2009, Yang *et al.* 2009), Chinese flowering cabbage (*B. campestris* L. ssp. *parachinensis*; Zhou *et al.* 2010a), and purple flowering stalk (*B. campestris* L. ssp. *chinensis* var. *purpurea* Hort.; Feng and Lou 2011). However, a multiple-allele male sterile line of Wutacai with similar traits as the target line and complete sterility has not yet been reported. In this research, the multiple-allele male sterility of Naibaicai was successfully transferred to Wutacai by utilizing a "directional transfer program." We bred a new

Table 9. Fertility identification of GMS-3 in the Wutacai

Male sterile line	Total flowers in bags	Number of seeds in pods	Sterility degree	Total plants	Number of fertility plants	Sterility rate
GMS-3	1000	0	100%	100	0	100%

Table 10. Plot yields of hybrid combinations in Wutacai

Code	Combination	Replication			Plot yield
		I	II	III	kg/4.5 m^2
CK	vitamin	10.80	9.00	10.80	10.20
C1	GMS-3 × P_1	15.54	14.31	14.61	14.82**
C2	GMS-3 × P_2	10.16	11.61	9.48	10.42
C3	GMS-3 × P_3	11.94	13.36	15.36	13.55*
C4	GMS-3 × P_4	16.86	15.41	15.15	15.81**

* Significant at the 5% level. ** Significant at the 1% level.

male sterile line, GMS-3, with 100% sterile plants and showing complete male sterility.

Directional transfer is crucial in the breeding of a male sterile line. Because the male sterile line of Wutacai itself was equivalent to a hybrid, the hybrid seed was actually a three-way crossing seed with such a male sterile line as the female parent (Wang *et al.* 2006). Thus, we focused on the transfer of both male sterility and botanical traits. Following the directional transfer program, the AB line and its "temporary maintainer" could be considered as siblines that were similar to the target line in most traits after backcrossing. As a result of similar genetic backgrounds, the male sterile line developed using this system is very stable for the inheritance of traits. In addition, the hybrid seeds, which were developed from this male sterile line, were of excellent uniformity.

A male sterility source with high quality and similar botanical traits to the target line are preconditions for developing an excellent male sterile line. This study also selected a subspecies of *B. campestris*, Naibaicai, as the male sterility resource. This plant's glossy dark green leaves have many folds. Naibaicai tastes fresh and crisp and has an abundance of nutritious components. Owing to many similarities between the male sterile resource and the target line, the breeding process was accelerated greatly.

The number of backcross generations is very important for the directional transfer effect. If the number of generations is too small, the effects of "directional" transfer cannot be achieved. However, if too many generations are involved, serious degeneration, such as self-incompatibility, petal deformity, or degeneration, may occur. Thus, during the directional transfer process, the number of backcross generations should be determined according to the degree of similarity in botanical traits between the backcross progeny and the recurrent parent. In this study, GMS-3, the male sterile line bred from the BC_4 generation, is almost the same as WT01 in terms of botanical traits and it does not show any degeneration. Backcrossing for four generations is an optimal choice for the directional transfer of male sterile lines in Wutacai.

The hybrids generated from GMS-3 as the female parent displayed a significant heterosis for yield, and the quality of hybrids achieved levels that are suitable for commercial production. Significant heterosis for yield was found in some hybrids. The C4 showed the highest plot yield, followed by C1, C3, and lastly C2. Compared with CK, their yields increased by 54.9%, 45.3%, 32.8%, and 2.2%, respectively. Similar heterosis in yield has been reported previously for other *Brassica* crops (Akshay *et al.* 1993, Dhirendra and Kumar 2012, Valiollah 2012, Wan *et al.* 2008). Analysis showed that the contents of chemical components in hybrid combinations of Wutacai were higher than those of common conventional varieties (Zhou *et al.* 2010b, 2011). Component contents of C1 were higher than those of CK in most indexes. Organic acid and crude fiber contents of C1 were lower than those of CK, which indicated that C1 tastes better than CK. Thus, considering both yield and quality, we report here that a superior hybrid combination, C1, has been developed (Fig. 1G, 1H).

Acknowledgments

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Table 11. Components analysis of hybrid combinations in Wutacai

	Soluble sugar g/100 g	Protein mg/100 g	Vc[a] mg/100 g	Organic acids %	Crude fiber %	K mg/100 g	P mg/100 g	Fe mg/100 g	Ca mg/100 g	Zn mg/100 g
CK	1.756	3.493	201.373	0.328	4.798	21.251	5.316	0.542	10.612	0.165
C1	1.761*	3.660**	229.342**	0.194	4.096	20.920	6.231**	0.291	10.824**	0.144
C2	1.570	3.464	186.117	0.176	5.916**	18.828	5.446**	0.331	10.498	0.370**
C3	1.750	3.591**	198.830	0.145	3.914	15.913	4.548	0.275	10.670**	0.115
C4	1.545	3.424	191.203	0.254	5.440**	13.409	6.510**	0.379	11.076**	0.320**

[a] Ascorbic acid.

* Significant at the 5% level. ** Significant at the 1% level.

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